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PATTERN OF VIRAL RNA SYNTHESIS IN A TEMPERATURE-SENSITIVE MUTANT OF EASTERN EQUINE ENCEPHALITIS VIRUS

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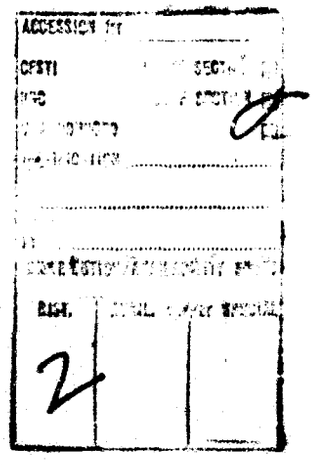

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PATTERN OF VIRAL RNA SYNTHESIS IN A TEMPERATURE-SENSITIVE MUTANT OF EASTERN EQUINE ENCEPHALITIS VIRUS

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Project 1C014501B71A

January 1968

ABSTRACT

The pattern of viral RNA synthesis of a temperature-sensitive mutant (Ets-4) of eastern equine encephalitis virus was compared with that of the wild type parent E at 37 C. The production of infectious Ets-4 virus was moderately inhibited at this temperature and strongly inhibited at 42 C, whereas the wild type reproduced equally well at both temperatures. At 37 C, the mutant produced excessive amounts of poorly infectious 18S and 28S species of viral RNA compared with the parent and nearly normal amounts of infectious 46S RNA. Based on recent evidence by other workers, our data suggest that a rate-limiting step occurred at the presumed conversion of 28S RNA to 46S RNA.

PATTERN OF VIRAL RNA SYNTHESIS IN A TEMPERATURE-SENSITIVE MUTANT OF EASTERN EQUINE ENCEPHALITIS VIRUS

This report describes a temperature-sensitive mutant virus strain derived from the Louisiana strain of eastern equine encephalitis (E) virus that appears to be unusual with respect to its pattern of viral RNA synthesis in chick embryo cells. The virus mutant, Ets-4, was induced by treatment of eastern equine encephalitis viral RNA with nitrous acid and was selected for its ability to grow well at low temperature (30 C) but poorly at 42 C. Comparisons of E and Ets-4 were always made at equal multiplicities of infectivity at about 10 to 50.

Both Ets-4 virus and its parent E multiply at similar rates at 30 C. However, as shown in Figure 1, their relative growth rates differ at higher temperatures. E virus multiplied equally well at 37 C and 42 C, but Ets-4 was moderately inhibited at 37 C and strongly inhibited at 42 C. These data show that Ets-4 is a temperature-sensitive mutant whose growth is inhibited significantly at temperatures greater than 37 C.

In light of the relatively poor growth of Ets-4, it was of interest to compare viral RNA synthesis of this virus with that of the parent strain by measuring the incorporation of uridine-C^{14}. It was surprising to find that this virus induces chick embryo infected cells to incorporate considerably more C^{14}-labeled uridine than the parent strain at 37 C.

The incorporation of uridine-C^{14} by Ets-4 and E virus-infected cells at 37 C and 42 C is shown in Figure 2. Actinomycin D at 2 μg/ml was added to the infected cultures to inhibit cellular RNA synthesis so that most of the incorporation of uridine by cells represented that induced by the virus. At 6 hours postinfection at 37 C, Ets-4 virus induced chick embryo cells to take up more than three times as much uridine as E virus; at 10 hours, the uridine uptake was twice as high in Ets-4 as in E. At 42 C the uridine uptake of Ets-4 was reduced sharply, but roughly approximated that of E virus at the same temperature. These results show that Ets-4 virus, which attained lower titers of mature virus than its parent, was nevertheless able to synthesize considerably more viral RNA.

It seemed possible that the maturation step involving the assembly of the protein and viral RNA into complete virus was defective and that, in this case, Ets-4 might be able to synthesize more infectious RNA than the parent strain. Cells infected with Ets-4 were tested for uridine-C^{14} uptake in the presence of actinomycin D. At the same time, the viral RNA was extracted with hot phenol and sodium dodecyl sulfate and assayed for infectious RNA on chick embryo cell monolayers made hypertonic with 1 M sodium chloride.

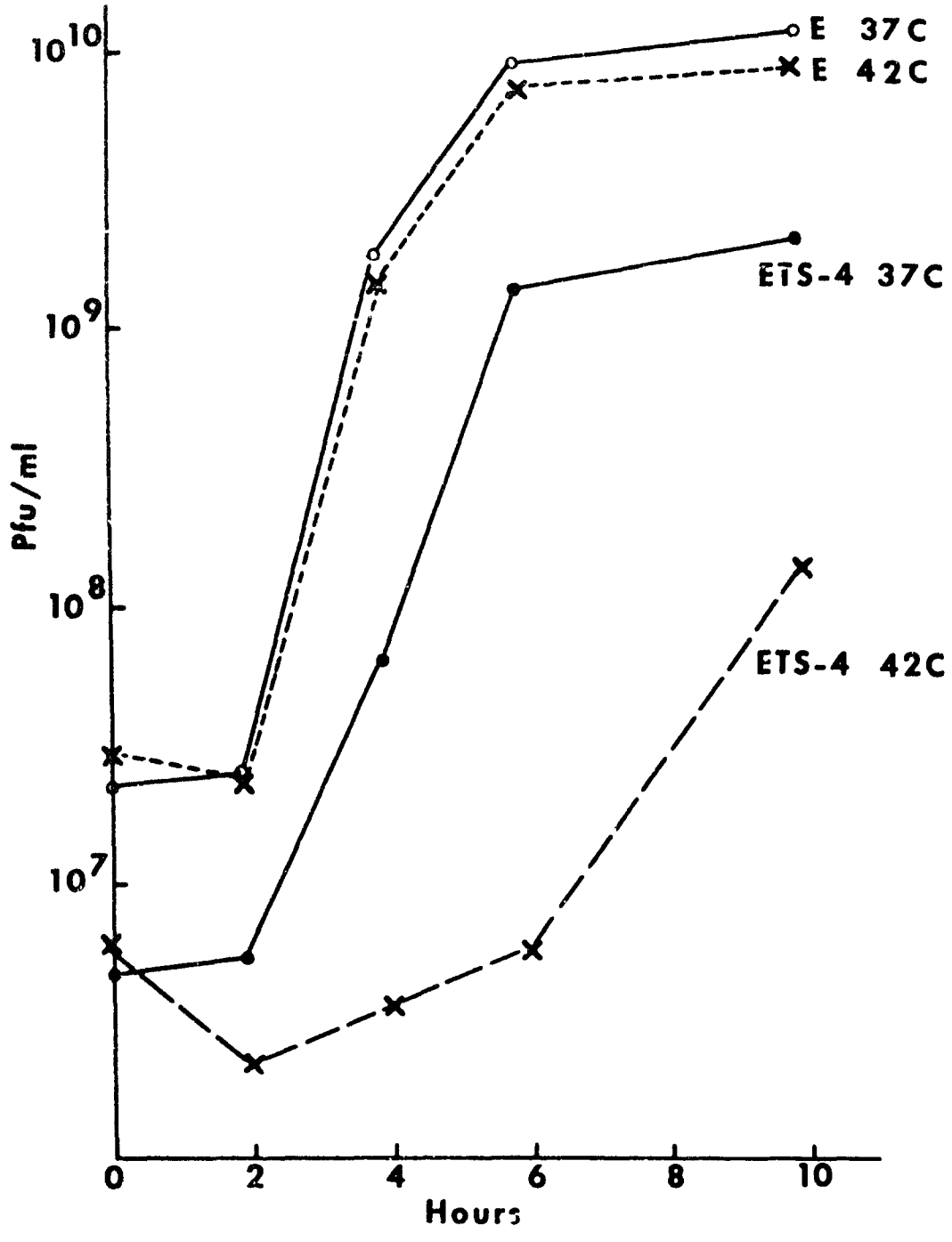

Figure 1. Growth Response of E and Ets-4 at 37 C and 42 C.

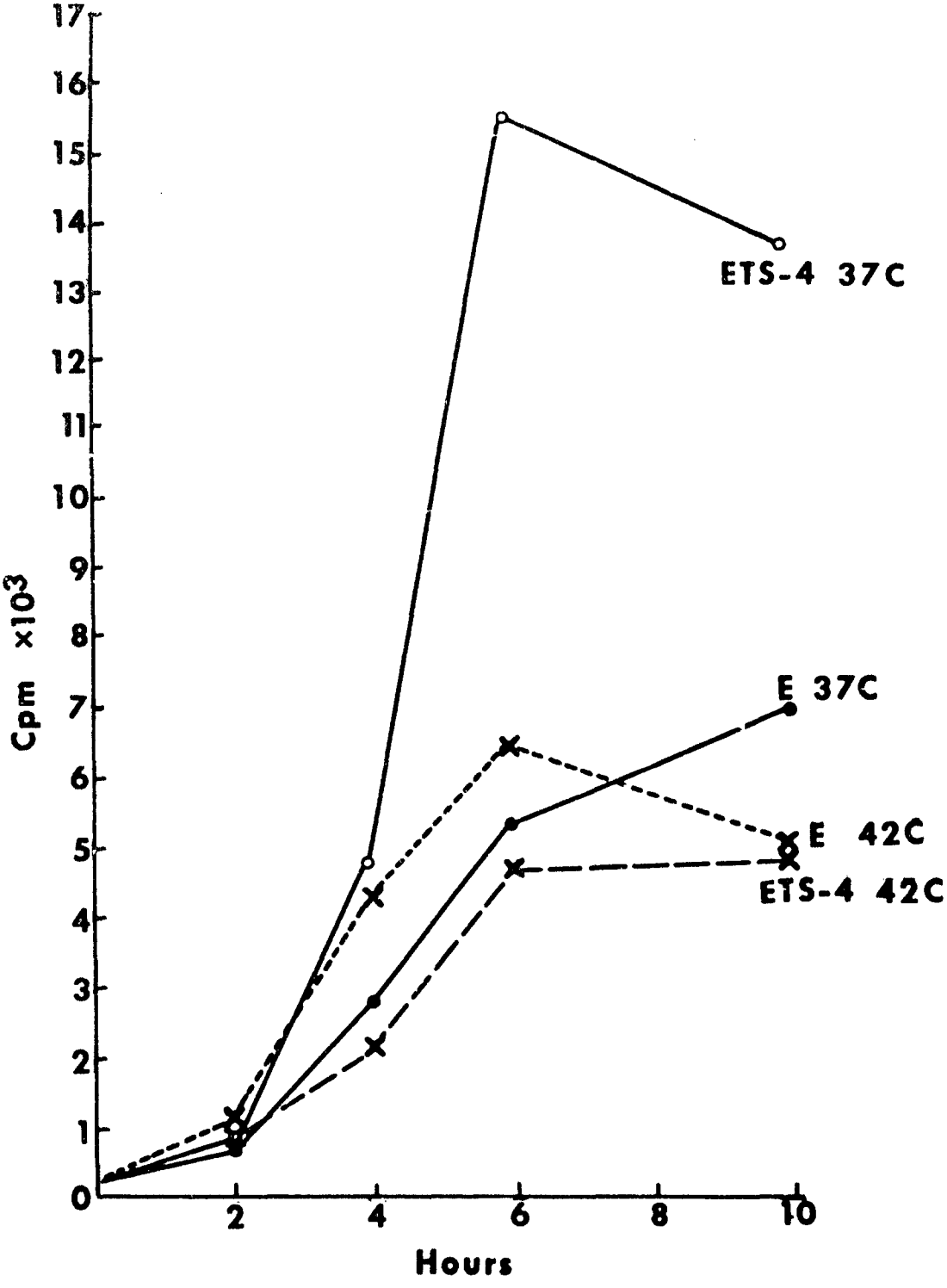

Figure 2. Uridine-2-C^{14} Uptake by E and Ets-4 Viruses at 37 C and 42 C.

Table 1 shows the results of this experiment. The infectious RNA titer of Ets-4 was only about one-third that of E, but the radioactivity incorporated into infected cells by Ets-4 was almost three times higher than that incorporated by E. To permit meaningful comparison of the data obtained for both viruses, the data in the third column of this table were expressed as specific activity, that is, as counts per minute per plaque-forming unit of infectious RNA. The values obtained for each virus were then expressed as a ratio of the specific infectivity of Ets-4 to that of E virus. The results show that for Ets-4, 10 times more uridine was incorporated for each unit of infectious RNA than for E. These results indicate that the increased viral RNA synthesis by Ets-4 did not result in a corresponding increased amount of infectious RNA and that most of the viral RNA synthesis resulted in poor quality nucleic acid products with respect to infectivity.

TABLE 1. RELATIONSHIP OF URIDINE-2-C^{14} UPTAKE BY INFECTED CELLS TO INFECTIOUS RNA FORMED BY E AND Ets-4 VIRUSES

Virus	Infectious RNA[a]	Cpm	Specific Activity, cpm/pfu	Ratio[b]
E	3.1×10^6	7,679	0.0025	-
Ets-4	9.0×10^5	23,318	0.026	10

a. Infectious RNA extracted by hot phenol - sodium dodecyl sulfate from approximately 1.0×10^8 chick embryo cells.

b. Ratio = $\frac{\text{specific activity of Ets-4}}{\text{specific activity of E}}$

In an effort to determine the nature of the viral RNA formed by Ets-4, an experiment was performed in which chick embryo cells infected with Ets-4 or E were grown in the presence of C^{14}-labeled uridine and actinomycin D for 5 hours. The RNA was extracted from the infected cells with hot phenol and sodium dodecyl sulfate and precipitated twice with ethyl alcohol. The RNA was dissolved in phosphate buffered saline, placed on a 15 to 35% sucrose gradient in a 30-ml tube, and centrifuged overnight at 22,000 rpm. Fractions of 10 drops each were collected, and the RNA was precipitated with trichloroacetic acid and collected on Millipore filters. The radioactivity trapped on the filters was measured with a liquid scintillation counter.

Figure 3 shows the sedimentation patterns of RNA from Ets-4 and E viruses. The positions of ribosomal RNA peaks from chick embryo cells were determined by optical density measurements. Arrows on this curve indicate 28S and 18S ribosomal RNA peaks. The peaks were used as reference points to calculate the sedimentation coefficient of the radioactive viral RNA peaks. E virus shows two major peaks: one at 46S, which is the infectious viral RNA and is identical to RNA extracted from purified virus, and a 28S RNA component. The 28S RNA has been observed for other arboviruses; its function in viral RNA synthesis is not known, but it is believed by some to be a precursor and/or a different form of the infectious viral RNA that sediments at 46S. The amount of total label incorporated into virus-induced RNA was considerably greater for Ets-4 than for E virus. Two major peaks were observed for Ets-4: a large 28S RNA peak and an 18S RNA component. The 18S RNA peak was resistant to 1 μg/ml ribonuclease for 10 minutes at room temperature and was considered to be a mixture of the double-stranded RNA and the replicative intermediate observed in several laboratories since Franklin's observations on RNA phage replication. The double-stranded RNA peak was seen as a shoulder in the curve for E virus, and it was located in the same position as observed for Ets-4 when the fractions were tested for ribonuclease resistance.

The 46S RNA peak was not observed as such for Ets-4, but it too was located in the same position as that of E virus, as indicated by infectious RNA assay of the fractions. It was probably obscured by the spread of the 28S RNA material in the sucrose gradient.

Table 2 was prepared with the data shown in Figure 3 for fraction 12, which contains the peak of the radioactivity representing the 46S RNA, and fractions 24 and 30 that have the 28S and 18S RNA.

Table 2 shows a comparison of the total radioactivity of the 46S infectious RNA and that of ribonuclease-resistant 18S and 28S RNA for E and Ets-4 viruses. The ratio of the 28S to 46S RNA for E was 1.6; that of Ets-4 was twice as great. The ratio of ribonuclease-resistant 18S RNA to 46S RNA was almost three times greater for Ets-4 virus.

These data confirm the substantial accumulation of the 18S and 28S RNA species in Ets-4-infected cells and suggest that the presumed conversion of the 18S and 28S to the 46S infectious RNA was less efficient for Ets-4 than for E virus.

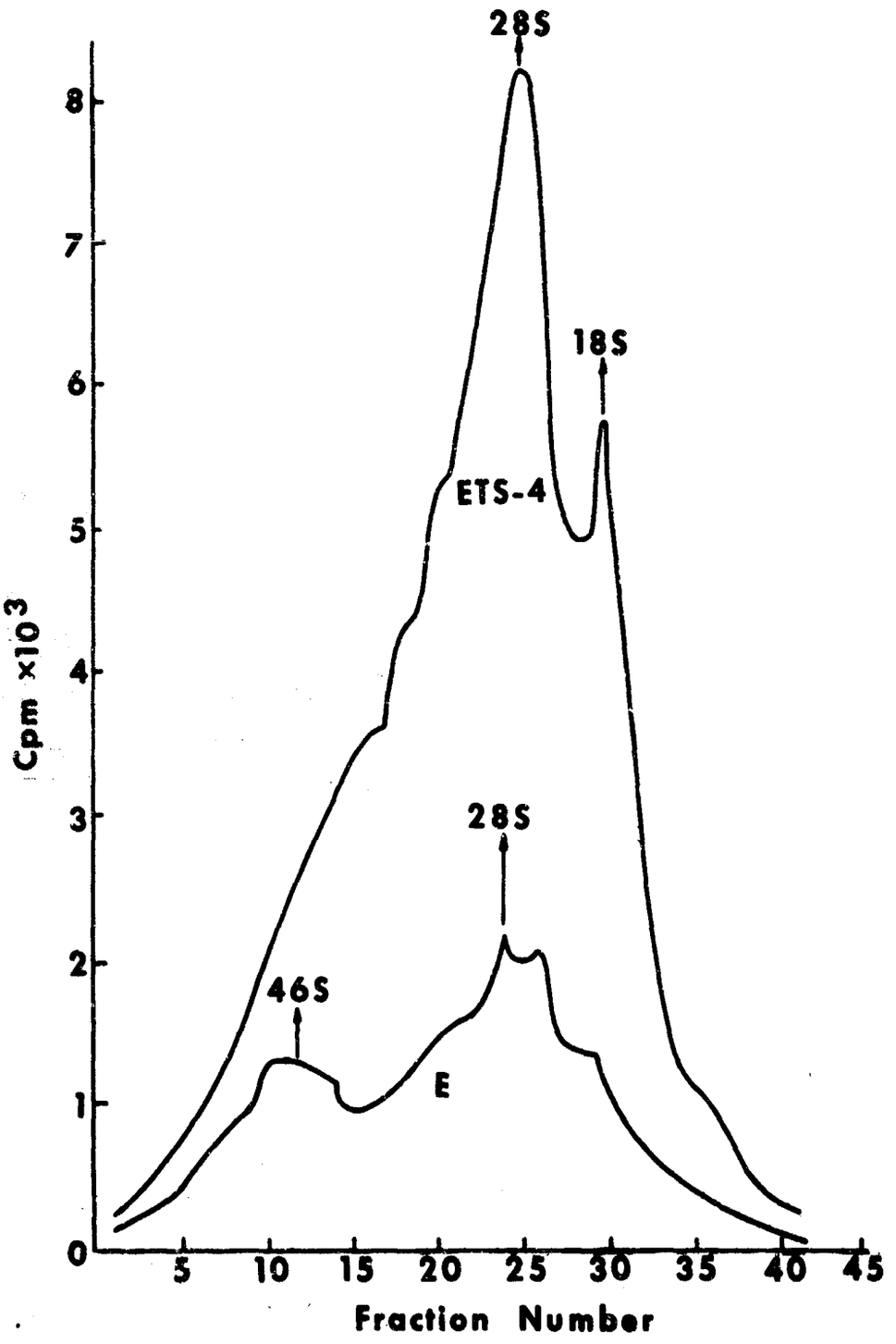

Figure 3. RNA Sedimentation Pattern of E and Ets-4 Virunes on Sucrose Gradient.

TABLE 2. RATIO OF 46S INFECTIOUS RNA TO 28S AND 18S OF E AND Ets-4 VIRUSES

Virus	RNA Species, cpm			Ratio	
	46S[a]	28S[b]	18S[c]	28S / 46S	18S / 46S
E	1,314	2,073	795	1.6	0.6
Ets-4	2,465	8,245	4,201	3.3	1.7

a. Peak count for 46S RNA (Fraction 12).
b. Peak count for 28S RNA (Fraction 24).
c. Peak count for 18S (Fraction 30) after treatment of fraction with 1 μg/ml ribonuclease for 10 minutes at room temperature.

The information available at this time indicates that Ets-4 induces excessive amounts of viral RNA, and that this RNA is largely the 18S and 28S species. Recent evidence from other laboratories, confirmed in preliminary pulse and chase experiments in our laboratory, supports the notion that RNA synthesis of arboviruses involves a sequence that includes conversion of the original genome to double-stranded and/or replicative intermediate forms (in the 20S to 16S region); a 28S form is then synthesized and this is followed by the appearance of the infectious 46S form found in the completed virus particle. In arboviruses, the RNA species that are less than 46S are known to be poorly infectious. If this total picture is correct, it appears that the rate-limiting step occurs in the conversion of 28S to the 46S form. One of several possible explanations of our results holds that, whatever the reason for the apparently derepressed or poorly controlled excess synthesis of 18S and 28S forms of viral RNA, the process (e.g., an enzyme) for converting 28S to 46S RNA is saturated and cannot handle the excess RNA. Another possible explanation is that the 28S RNA is defective and cannot be converted to 46S; still a third more unlikely possibility, in our view, is that the rate-limiting step itself is the site of a defect and, therefore, the cause of the excessive accumulation of the other RNA species.

Experiments are under way to study these and other possible explanations for the unusual pattern of RNA synthesis of Ets-4 virus.

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13. ABSTRACT

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